

Effect of anemia and renal cytokine production on erythropoietin production during blood-stage malaria

KAI-HSIN CHANG and MARY M. STEVENSON

Institute of Parasitology, Macdonald Campus, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada; Centre for the Study of Host Resistance, McGill University Health Centre Research Institute, McGill University, Montreal, Quebec, Canada

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Background. Renal dysfunction and severe anemia are clinical complications of blood-stage malaria. Erythropoietin (Epo) is a hormone produced by the kidney and plays an essential role in stimulating erythrocyte production. Renal dysfunction in malaria is associated with changes in renal cytokine levels, which may affect the production of Epo and the alleviation of anemia.

Methods. Resistant C57BL/6 (B6) and susceptible A/J mice were infected with *Plasmodium chabaudi* AS. The levels of Epo and cytokines were measured by enzyme-linked immunosorbent assay (ELISA) and the degree of anemia was determined by hematocrit. Regression analyses were employed to estimate the influences of anemia and renal cytokines on the production of Epo during infection.

Results. A/J mice developed higher peak parasitemia, more severe anemia, and succumbed as compared to B6 mice, which survived the infection. B6 mice had higher levels of renal tumor necrosis factor- α (TNF- α) and interleukin (IL)-10, whereas A/J mice had higher levels of IL-12p70, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, and Epo. Regression analyses revealed that kidney Epo levels were influenced most strongly by changes in hematocrit levels. In addition, albeit to a much weaker degree, kidney Epo levels correlated negatively with GM-CSF levels but positively with IL-10 levels.

Conclusion. Blood-stage malaria infection modulates the production of renal pro- and anti-inflammatory cytokines in resistant versus susceptible strains of mice differentially. However, despite the fluctuations of renal cytokines, the degree of anemia is the main determinant for Epo production during blood-stage malaria while kidney cytokines may exert secondary influences.

Malaria is the most serious parasitic disease and causes over 300 million cases and 1 to 2 million deaths each year in endemic areas. Severe anemia is one of the major life-threatening manifestations of malaria, especially among

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children under the age of 5 years and pregnant women in sub-Saharan Africa. The cause of severe malarial anemia is multifactorial. Direct destruction of erythrocytes by the *Plasmodium* parasite and shortened life span of uninfected erythrocytes are considered to be leading causes of anemia during malaria [1]. Furthermore, inappropriately low reticulocytosis relative to the degree of anemia suggests insufficient erythropoiesis during malaria [2, 3]. The etiology of insufficient erythropoiesis during malaria is, however, not fully understood. One proposed mechanism is inadequate erythropoietin (Epo) production [4, 5].

Epo is the essential growth factor that promotes erythropoiesis by supporting the proliferation, differentiation, and maturation of erythroid precursors. Unlike other hematopoietic growth factors, Epo is produced primarily in the kidneys instead of in the bone marrow where target erythroid precursor cells reside. In the kidney, the site of Epo production is localized to the peritubular fibroblasts of the renal cortex [6–8]. It is well acknowledged that tissue oxygen tension regulates Epo production in a feedback loop, which translates into an inverse logarithmic relationship between plasma Epo and hematocrit levels [9]. However, in anemia due to renal failure, chronic inflammation, and cancer, Epo production is not adequate. Several proinflammatory cytokines have been demonstrated to inhibit Epo production, including tumor necrosis factor- α (TNF- α), interleukin (IL)-1, and IL-6 [10, 11].

Fluctuation of proinflammatory and anti-inflammatory cytokine levels in the kidney has been reported in malaria-related renal disorders. Malaria causes acute interstitial inflammation in rodents [12]. An increase in serum TNF- α is associated with renal failure in human malaria due to *P. falciparum* [13]. Immunohistochemistry revealed that increased staining for TNF- α , IL-1 α , IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and decreased staining for IL-10 are associated with malarial tubulointerstitial nephritis in rodents [14]. On the other hand, murine malarial glomerulonephritis is associated with increased

messenger RNA levels of TNF- α , IL-1 α , IL-6, and IL-10 [15]. Although dysregulation of renal cytokine production during malaria is well-documented, the link between cytokine and Epo production in the kidney is not clear. The purpose of this study was to characterize the development of malarial anemia and the kinetics of kidney cytokine production, and to analyze their relationship with Epo production in the murine model of *P. chabaudi* AS infection. Using regression analysis, we demonstrate that during the acute phase of blood-stage *P. chabaudi* AS infection in mouse strains that are either resistant or susceptible, Epo production is regulated primarily by the degree of anemia whereas fluctuations in kidney cytokine levels play a less prominent role.

METHODS

Experimental infection and animals

A/J mice, 8 to 10 weeks old, were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and C57BL/6 (B6) mice were purchased from Charles River Laboratories (St. Constant, Quebec, Canada). Mice were age- and gender-matched in all experiments. *P. chabaudi* AS parasites were maintained as previously described [16]. Mice were infected intraperitoneally with 10^6 parasitized red blood cells. Mice were sacrificed at the designated time points and blood was collected. Blood smears were prepared and stained with Diff-Quik[®] Solution (Dade Behring, Duding, Switzerland), and parasitemia was determined by counting at least 400 cells. Furthermore, cardiac puncture was performed and 70 μ L of blood were obtained for determination of hematocrit levels. For each sample, the remaining blood was allowed to clot at room temperature and centrifuged twice at $1000 \times g$ for 10 minutes at room temperature for serum collection. Sera were stored at -20°C until tested for Epo concentrations. Kidneys were harvested, snap-frozen in liquid nitrogen, and stored at -70°C until tissue processing. All animal manipulations were done in accordance with the animal care guidelines implemented by the McGill University Health Centre Research Institute.

Kidney homogenization

Kidneys were thawed in 2 mL sterile phosphate-buffered saline (PBS) and homogenized three times for 15 seconds with a Brinkmann homogenizer (Brinkmann Instruments, Mississauga, Ontario, Canada). Kidney homogenates were then subjected to three freeze-thaw cycles at -70°C for 4 hours and 37°C for 15 minutes. Supernatants were separated from tissue residues by centrifugation twice at $12,000g$ for 10 minutes at 4°C .

Determination of protein, Epo, and cytokine concentrations

Total protein concentrations in supernatants of kidney homogenates were measured using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer's instructions. Epo concentrations in supernatants of kidney homogenates and sera were measured by a murine Epo-specific enzyme-linked immunosorbent assay (ELISA) recently developed in our laboratory [17]. Monoclonal rat anti-mouse Epo antibody (BD BioSciences, Mississauga, Ontario, Canada) and polyclonal rabbit antihuman Epo antibody (R&D Systems, Minneapolis, MN, USA) were used as capturing and detecting antibody, respectively. Recombinant murine Epo was purchased from Boehringer Mannheim-Roche (Laval, Quebec, Canada). Levels of TNF- α , interferon- γ (IFN- γ), IL-12p70, GM-CSF, IL-10, and IL-4 in supernatants of kidney homogenates were determined by sandwich ELISA using paired capture and detection antibodies as previously described [18–20]. Reactivity was revealed using ABTS substrate (Boehringer Mannheim-Roche) and optical density (OD) values were read at a wavelength of 405 nm with 492 nm as reference. The concentrations of cytokine in the samples were calculated against standard curves generated using recombinant cytokines (BD PharMingen, Mississauga, Ontario, Canada) and expressed as per mg of renal protein. Renal IFN- γ was undetectable and thus was not included in statistical analyses.

Statistical analyses

Values are expressed as mean \pm standard error of the mean (SEM). The effects of mouse strain on the course of blood-stage *P. chabaudi* AS infection were determined with repeated measures analysis of variance (ANOVA). Statistical significance of differences in Epo and cytokine levels between A/J and B6 mice were analyzed by Student *t* test. A $P < 0.05$ in the *t* test was considered to be significant. Kidney and serum Epo levels were logarithmically transformed before performing correlation or regression analyses. Pearson correlation coefficient analysis was performed to determine the association between two variables of any combinations. The hypothesis that two variables are uncorrelated was tested and a $P < 0.05$ was considered to be significant. Log-transformed kidney Epo levels were then used as the dependant variable in multivariate regression analysis. Hematocrit levels and the levels of cytokines at all time points were included as independent variables. The forward selection method of multiple regression analysis was used to determine the regression coefficient and partial R^2 of each variable. Forward selection with significance level set at 0.15 for the *F* test was used to identify variables having significant influence on the variability of kidney Epo levels. Stepwise selection that combined forward selection and backward

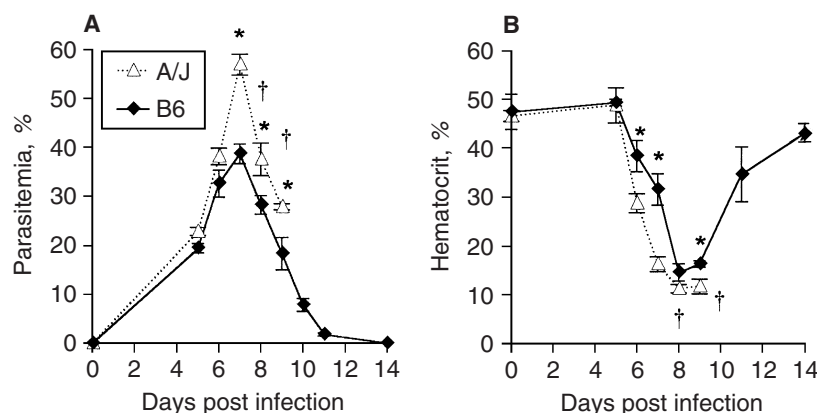


Fig. 1. Course of parasitemia and progression of anemia during *Plasmodium chabaudi* AS infection in resistant C57BL/6 (B6) and susceptible A/J mice. Course of parasitemia (A) and hematocrit levels (B). Values are mean \pm standard error of the mean for three to six mice per group. * $P < 0.05$, A/J vs. B6 mice. † Indicates mortality. Similar results were obtained in four independent experiments.

elimination was also employed and the same subsets of variables were identified as significant by forward selection. All analyses were performed using SAS/STAT software (SAS Institute, Cary, NC, USA).

RESULTS

Course of *P. chabaudi* AS infection in B6 and A/J mice

Following infection with *P. chabaudi* AS, parasite replication was accelerated on day 5 postinfection and parasitemia peaked on day 7 postinfection in both resistant B6 and susceptible A/J mice (Fig. 1A). All mice developed anemia, which worsened following peak levels of parasitemia (Fig. 1B). A/J mice had significantly higher peak parasitemia, suffered earlier onset and more severe anemia, and succumbed by day 8 to 9 postinfection when they were most anemic. In contrast, B6 mice controlled acute infection by 2 weeks, and their hematocrit levels began to increase by day 9 to 11 postinfection and returned to 85% of preinfection normal level by day 14 postinfection.

Renal proinflammatory and anti-inflammatory cytokine production during *P. chabaudi* AS infection

The kidneys of both infected B6 and A/J mice were discolored and appeared puffy with edema as compared to those collected from normal mice (unpublished personal observations). Determination of cytokine levels in the supernatants of kidney homogenates by ELISA revealed that infection led to increased TNF- α production in both resistant B6 and susceptible A/J mice (Fig. 2A). B6 mice had a significantly higher level of renal TNF- α on day 6 postinfection as compared to A/J mice. Renal TNF- α production in B6 mice peaked on day 8 postinfection and remained significantly higher than baseline levels until day 14 postinfection ($P < 0.05$ for days 8 to 14 postinfection vs. day 0 postinfection). For A/J mice, the increase in renal TNF- α was delayed in comparison to B6 mice and reached the peak level on day 9 postinfection when mortality occurred. However, there were no signif-

icant differences in peak TNF- α levels between B6 and A/J mice.

In contrast to the kinetics of renal TNF- α production, the increases in renal IL-12p70 level were transient in both strains of mice, and peaked on day 5 postinfection (Fig. 2B). A secondary peak was also observed in both strains of mice, which occurred on day 8 postinfection in A/J mice and on day 11 postinfection in B6 mice. The level of IL-12p70 in kidney was significantly higher in A/J than B6 mice on day 8 postinfection. Renal GM-CSF production followed a similar pattern of kinetics in A/J and B6 mice (Fig. 2C). Both strains had a primary peak of GM-CSF on day 5 postinfection, followed by a secondary peak which occurred on day 8 postinfection in A/J mice and on day 11 postinfection in B6 mice. There was a significant difference between A/J and B6 mice on day 8 postinfection.

In addition to up-regulated production of proinflammatory cytokines in the kidneys, production of the anti-inflammatory cytokine, IL-10, was also transiently increased (Fig. 2D). There were no significant differences throughout the infection between B6 and A/J mice except on day 5 postinfection, when the renal IL-10 level was significantly higher in B6 compared to A/J mice.

The levels of IL-4, the signature Th2 cytokine, were also measured. Primary and secondary peaks were observed on day 5 and day 11 postinfection, respectively, in B6 mice (Fig. 2E). However, due to high within-group variability in B6 mice, there were no significant differences when comparing these time points with the baseline level at day 0. In contrast, A/J mice had two peaks of renal IL-4 production on day 5 and day 8 postinfection, and the levels at both time points were significantly higher in comparison to the day 0 baseline level ($P < 0.05$ day 5 postinfection vs. day 0 postinfection, $P < 0.05$ day 8 postinfection vs. day 0 postinfection). By day 9 postinfection, when mortality occurred in A/J mice, the IL-4 level was still significantly higher compared to baseline ($P < 0.05$). When comparing A/J to B6 mice, A/J mice had a significantly higher level of renal IL-4 on day 8 postinfection.

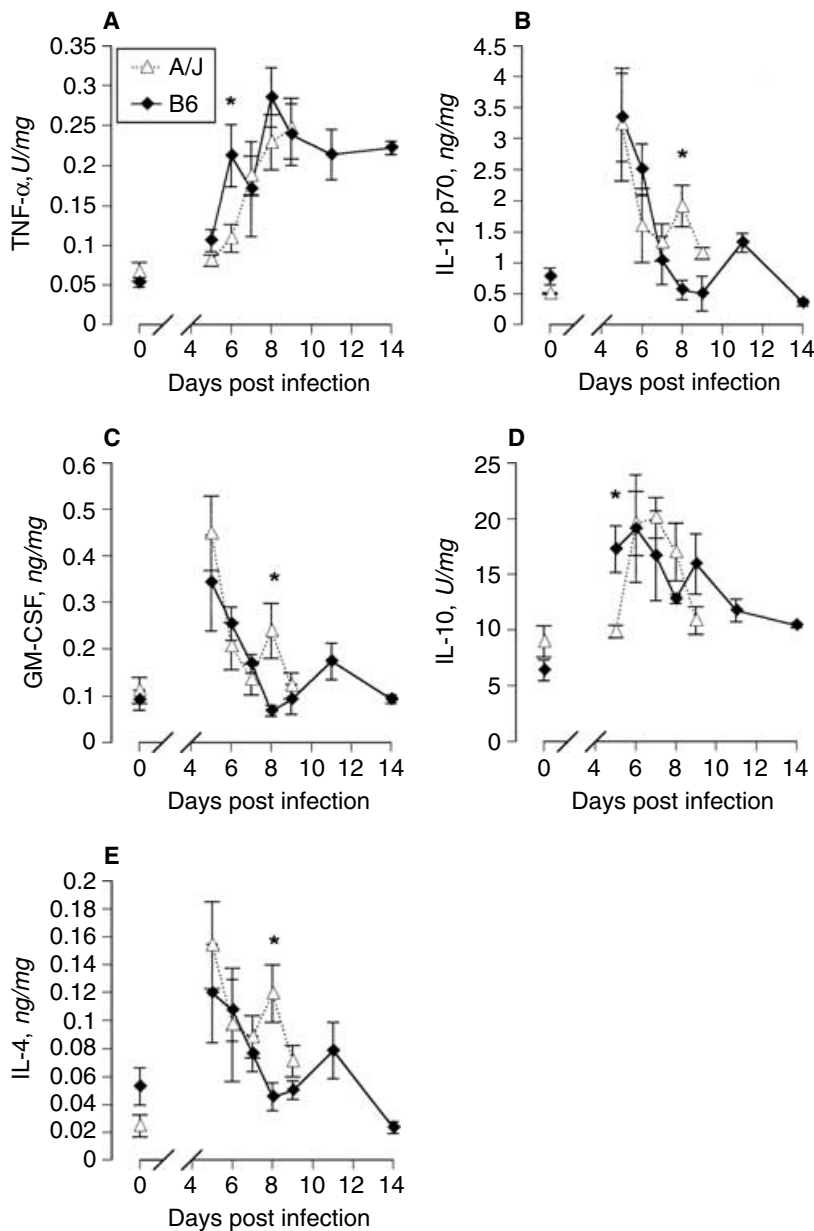


Fig. 2. Kinetics of renal cytokine production during *Plasmodium chabaudi* AS infection in resistant C57BL/6 (B6) and susceptible A/J mice. Levels of tumor necrosis factor- α (TNF- α) (A), interleukin (IL)-12p70 (B), granulocyte-macrophage colony-stimulating factor (GM-CSF) (C), IL-10 (D), and IL-4 (E) in the supernatants of kidney homogenates. Values are mean \pm standard error of the mean for three to six mice per group. * $P < 0.05$, A/J vs. B6 mice. Similar results were obtained in two independent experiments.

Levels of renal and serum Epo during *P. chabaudi* AS infection

Epo concentrations in the supernatants of kidney homogenates and sera were measured by ELISA. As presented in Figure 3, there were significant increases in Epo production in the kidneys of both mouse strains following the development of malarial anemia on days 6 and 7 postinfection (Fig. 3A). Consistent with earlier onset and more severe anemia, significantly higher levels of Epo were detected in kidney homogenates of A/J mice, which reached a peak level on day 9 postinfection when these mice were most anemic. In contrast, increased Epo production was transient in B6 mice and Epo levels returned to baseline when anemia was alleviated by days 11 to 14

postinfection. The concentration of Epo in serum samples (Fig. 3B) showed similar trends as those detected in kidney homogenates. A/J mice had significantly higher levels of serum Epo levels on days 6, 7, and 9 postinfection compared to B6 mice. Correlation analysis revealed that serum Epo levels were significantly correlated with kidney Epo levels (correlation coefficient $r_s = 0.88$ for A/J, $P < 0.001$; $r_s = 0.83$ for B6, $P < 0.001$).

Correlation analyses among kidney Epo levels, degree of anemia, and renal cytokine production

Univariate analysis. Among kidney cytokines, simple correlation analysis showed that the production of IL-12p70, GM-CSF, and IL-4 strongly correlated with

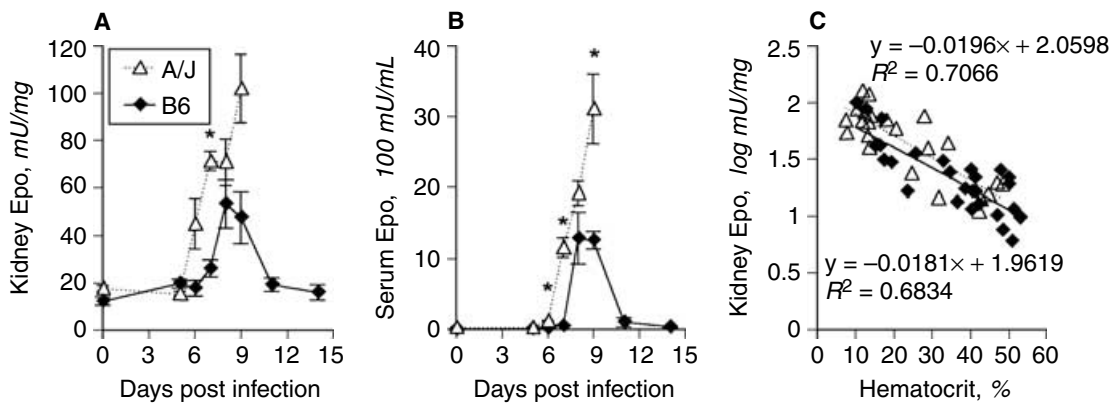


Fig. 3. Kinetics of erythropoietin (Epo) levels during *Plasmodium chabaudi* AS infection in resistant C57BL/6 (B6) and susceptible A/J mice. Epo levels in kidneys (A), Epo levels in sera (B), and regression analysis (C) for relationship between kidney Epo levels and hematocrit levels. Values are means \pm standard error of the mean for three to six mice per group per time point except for (C) in which $N = 27$ for B6 mice and $N = 24$ for A/J mice. * $P < 0.05$, A/J vs. B6 mice. Similar results were obtained in replicate experiment.

Table 1. Correlation coefficient (r_s) of association between two variables among renal erythropoietin, hematocrit, and renal cytokine levels in univariate analysis^a

	Epo	TNF- α	IL-12p70	GM-CSF	IL-10
B6 Mice ($N = 27$)					
Hematocrit	-0.83 ^b				
TNF- α	0.60 ^b				
IL-12p70	-0.29	-0.34			
GM-CSF	-0.36	-0.42 ^b	0.96 ^b		
IL-10	0.38 ^b	0.18	0.32	0.26	
IL-4	-0.23	-0.34	0.92 ^b	0.89 ^b	0.32
A/J Mice ($N = 24$)					
Hematocrit	-0.84 ^b				
TNF- α	0.69 ^b				
IL-12p70	-0.14	-0.14			
GM-CSF	-0.38	-0.34	0.78 ^b		
IL-10	0.52 ^b	0.30	0.11	-0.23	
IL-4	-0.05	-0.17	0.87 ^b	0.72 ^b	0.16

Abbreviations are: B6, C57BL/6; Epo, erythropoietin; TNF- α , tumor necrosis factor- α ; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor.

^a Pearson correlation coefficient analysis was performed to determine the association between two variables of various combinations. The hypothesis that two variables are not correlated was tested and $P < 0.05$ was considered to be significant.

^b $P < 0.05$.

Table 2. Regression coefficients and partial R^2 of individual variables derived from multivariate regression analysis^a

	B6 ($N = 27$)		A/J ($N = 24$)	
	Regression coefficient	Partial R^2	Regression coefficient	Partial R^2
Hematocrit	-0.018	0.683 ^b	-0.021	0.707 ^b
TNF- α	-0.293	0.003	-0.443	0.005
IL-12p70	0.137	0.018	0.047	0.002
GM-CSF	-0.859	0.013	-0.834	0.142 ^b
IL-10	0.015	0.063 ^b	0.006	0.018 ^b
IL-4	-1.984	0.011	-0.981	0.003

Abbreviations are: B6, C57BL/6; TNF- α , tumor necrosis factor- α ; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor.

^a Log-transformed kidney erythropoietin levels were used as the dependant variable. Multivariate regression analysis with forward selection was performed to determine regression coefficient and partial R^2 for individual variables.

^b Indicates variable meets the 0.15 significance level for the F tests used to judge for entry into the forward selection methods of multivariate regression analysis model.

each other in both B6 and A/J mice (Table 1). However, none of these three cytokines showed significant correlation with Epo production in either mouse strain in univariate correlation analysis. Surprisingly, Epo production strongly correlated with renal TNF- α levels and modestly correlated with IL-10 levels in both mouse strains. The strongest associations found were negative correlations between Epo production and hematocrit levels in B6 as well as A/J mice. As shown in Fig. 3C, the regression lines were not significantly different between B6 and A/J mice.

Multivariate analysis. To delineate the potential contribution of each variable to kidney Epo levels while adjusting for interactions among variables, multiple re-

gression analysis was conducted with forward selection to add in variables one at a time. When the levels of hematocrit, TNF- α , IL-12p70, GM-CSF, IL-10, and IL-4 were all included in the regression model, kidney Epo was negatively associated with hematocrit, TNF- α , GM-CSF, and IL-4, while positively associated with the level of IL-12p70 and IL-10 in both strains of mice (Table 2). Determination of partial R^2 contributed by individual variables suggested that hematocrit level was the single most important variable in predicting kidney Epo level. With the level of significance for the F test set at 0.15, two cytokines were found to be significantly associated with kidney Epo level in A/J mice. GM-CSF was negatively associated and IL-10 was positively associated with kidney Epo level. In B6 mice, IL-10 was the only cytokine significantly associated with kidney Epo level. TNF- α , IL-12p70, and IL-4 did not significantly correlate with kidney Epo level in either mouse strain.

DISCUSSION

The major symptoms during blood-stage malaria are associated with the replication of the *Plasmodium* parasites inside host red cells and the subsequent destruction of the red cells as the parasites mature. The clinical complications caused by *Plasmodium* parasites are not restricted to hematologic disorders. In addition to anemia, malaria infection can cause a full spectrum of clinical manifestations, which may involve other organs such as brain [21], lung [22], and kidney [12, 14, 15, 23]. Although numerous studies have been conducted to investigate the pathophysiologic impact of malaria on specific organs, the potential effect of changes in one organ on another tissue during infection has rarely been investigated. In this context, the kidney is an essential tissue not only for regulating the balance of water, sodium, potassium, and calcium, but also for maintaining constant red cell volume by producing Epo, the principal growth factor that promotes erythropoiesis. When anemia occurs, decreased tissue oxygen tension up-regulates Epo production in the kidney. Epo is then transported via the circulation to hematopoietic tissues to promote erythropoiesis. The net effect of this cascade of events is alleviation of anemia. As anemia is a frequent complication among malaria patients and also occurs in experimental animal models, kidney dysfunction during blood-stage malaria may have a profound effect on the outcome of malarial anemia.

In this study, we characterized the kinetics of proinflammatory and anti-inflammatory cytokine production in the kidney during acute blood-stage *P. chabaudi* AS infection. Consistent with the results of studies on nephritis associated with *P. berghei* ANKA infection in mice [14, 15], we identified increased levels of renal TNF- α following infection. However, increased renal TNF- α production was not associated with fatal outcome of the infection as resistant B6 mice produced more TNF- α on day 6 postinfection in comparison to susceptible A/J mice. We also observed an increase in the levels of renal GM-CSF in both B6 and A/J mice. This cytokine has been proposed to be responsible for mesangial cell proliferation and infiltration of pigmented macrophages into the glomeruli during malaria infection [15]. In addition, increased levels of IL-10 were detected in both resistant B6 and susceptible A/J mice. The role of IL-10 in the pathogenesis of malarial nephritis is unclear. Previous studies indicate that IL-10 may contribute to tubulointerstitial nephritis and glomerulonephritis during murine malaria [14, 15]. Additionally, we observed increased production of IL-12p70 in both strains of mice, and of IL-4 in A/J mice. Increased production of IL-12p70 and IL-4 in the kidney during malaria has not been described previously. However, it has been suggested that IL-12 causes renal injury in crescentic glomerulonephritis and that increased serum IL-4 is associated with the development

of lupus nephritis [24, 25]. The presence of IL-12 and IL-4 in the kidney during malaria may cause renal injury in a similar fashion, possibly by acting on the infiltrating inflammatory cells, including CD4+ and CD8+ T cells and macrophages [12].

Cytokine regulation of Epo production is not fully understood, partially due to the difficulty of culturing Epo-producing renal cells. An in vitro study performed by Jelkmann et al [10] using kidney perfusion suggests that IL-1, IL-6 and TNF- α suppress Epo production. Furthermore, treatment of cancer patients with TNF- α has been linked to decreased Epo production [11]. However, the in vivo effect of increased cytokine production in the kidney on Epo production during disease states has never been investigated. Univariate analysis of our data in the *P. chabaudi* AS model revealed an inverse relationship between the levels of kidney Epo and hematocrit. Our data are consistent with the paradigm of feedback regulation of Epo production by red cell volume [9]. Our data are also consistent with the results of other malaria-related studies despite the controversy regarding the adequacy of Epo production relative to the degree of malarial anemia [4, 5, 16, 26–28].

In contrast to the proposed inhibition of Epo production by TNF- α [10, 11], univariate analysis showed a positive correlation between renal TNF- α and Epo levels during malaria in the present study. In addition, a positive association was also observed between renal IL-10 and Epo levels. As multiple factors may be involved in the regulation of Epo production during malaria, multivariate analysis was then conducted to take into consideration the interaction among hematocrit level and the levels of proinflammatory and anti-inflammatory cytokines. Hematocrit level was demonstrated to be the strongest variable in determining kidney Epo production during *P. chabaudi* AS infection in both resistant B6 and susceptible A/J mice. When the influence of hematocrit level was taken into account, TNF- α no longer correlated positively with kidney Epo level. In fact, a weak negative correlation was found between the levels of renal TNF- α and Epo, but it did not reach statistical significance. The lack of a strong inhibitory effect of TNF- α as suggested by the study of Jelkmann et al [10] may be due to the relatively low concentration of TNF- α detected in our study in comparison to the massive dose of exogenous TNF- α used in kidney perfusion in vitro. It is also possible that the TNF- α -producing renal cells may not be located in close enough proximity to Epo-producing cells to exert their inhibitory effect. However, multivariate analysis revealed that GM-CSF levels negatively correlated with levels of Epo in A/J but not B6 mice. GM-CSF may be responsible for infiltration of macrophages into the glomeruli during malaria infection [15], but its potential inhibitory effect on Epo production requires further investigation. In addition, the positive correlation between renal IL-10 and Epo levels, revealed by univariate

analysis, remained valid in multivariate analysis in both strains of mice. Based on these analyses, we postulate that IL-10 may have a stimulatory effect on Epo production. However, IL-10 appears to exert less influence on Epo production in comparison to the strong influence of hematocrit. The role of IL-10 in Epo production during blood-stage malaria can be further investigated using IL-10^{-/-} mice.

Thus, our study revealed a significant and strong effect of hematocrit level and a weak influence of proinflammatory and anti-inflammatory cytokines on Epo production during malaria infection. This explains, at least partially, the lack of significant differences in the logarithmic inverse relationships between Epo level and the degree of anemia in resistant B6 mice versus susceptible A/J mice, despite the differences in their kidney cytokine production profile. Because comparable levels of Epo relative to the degree of anemia were produced in both A/J and B6 mice during infection, insufficient erythropoiesis in A/J mice observed in our previous study [16] was not due to differential Epo production. Taken together, our data demonstrate that the acute phase of blood-stage malaria infection causes an increase in proinflammatory and anti-inflammatory cytokine production in the kidney. Our data also suggest that during blood-stage malaria infection, Epo production is strongly regulated by the degree of anemia. In addition, production of kidney cytokines, especially IL-10, and in susceptible A/J mice, GM-CSF, exerts minor influences.

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Reprint requests to Dr. Mary M. Stevenson, Centre for the Study of Host Resistance, McGill University Health Centre Research Institute, Room L11-409, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4.

E-mail: mary.m.stevenson@mcgill.ca

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